Characterization of Fluorescence in the Marine Environment

Charles Mazel
Physical Sciences Inc.,
20 New England Business Center,
Andover,
MA 01810
phone: (978) 689-0003 fax: (978) 689-3232 email: mazel@psicorp.com

Award Number: N0001402C0130

LONG-TERM GOALS

This is an exploration project aimed at documenting fluorescence of seafloor marine organisms. We wish to determine the nature and distribution, both geographic and taxonomic, of the phenomenon. The data gained has potential application to mapping and assessment of the sea floor.

OBJECTIVES

The objectives of the FY03 effort were to conduct fieldwork at several locations; refine techniques for photographing and videotaping fluorescence; make laboratory measurements of fluorescence properties; create a database of observations, images, and measurements; conduct deepsea fluorescence exploration from manned submersibles; and experiment with approaches to automated interpretation of multispectral fluorescence data.

APPROACH

This is largely a field-oriented project, supplemented by laboratory measurement and documentation. We are conducting SCUBA dives at a variety of locations, using several different imaging/exploration techniques to locate instances of seafloor fluorescence. We are concentrating on effects not previously observed or documented, by concentrating on habitats that have not been explored much or at all, and on taxonomic groups that have not been examined in detail. In this fiscal year a significant effort was devoted to outfitting a manned submersible for fluorescence search and documentation, and carrying out a series of deep exploratory dives. This part of the effort is being carried out by the Principal Investigator and by Dr. David Zawada.

When possible we collect specimens for laboratory measurement of fluorescence excitation and emission spectra. We are organizing our findings in a master database that incorporates observational information (identification, location, depth, appearance, etc.), white-light and fluorescence imagery, and spectral measurements.

We are developing approaches to computer interpretation of multispectral fluorescence data. These may be applied to several different means of collecting fluorescence data (e.g., photography, video, laser line scan) and are intended to produce robust approaches to the interpretation that are relatively immune to variations in sensor-to-subject distance and in-water optical properties. This is being done by generating sets of spectra that are based on our existing measurements of representative

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
30 SEP 2003		

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Characterization of Fluorescence in the Marine Environment	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Physical Sciences Inc.,,,20 New England Business Center,,Andover,,MA, 01810**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES
The original document contains color images.

14. ABSTRACT
This is an exploration project aimed at documenting fluorescence of seafloor marine organisms. We wish to determine the nature and distribution, both geographic and taxonomic, of the phenomenon. The data gained has potential application to mapping and assessment of the sea floor.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**		**7**	

fluorescence emission spectra (collected during this project and from prior projects such as CoBOP) from a variety of organisms divided into 15 types. We create a large number of variations of each of the 15 spectral classes by mathematically propagating the emission through varying viewing ranges and incorporating the effects of attenuation and spherical spreading. The resulting spectra are then divided into training and test data sets. We are experimenting with approaches including neural nets and simulated broadband detectors that use an approach along the lines of the animal visual systems comprising three or more overlapping photoreceptors. This work is being done by the Principal Investigator in collaboration with Jim Glynn and Don Frankel at PSI, both of whom have extensive experience with neural nets and algorithm development.

The project is a collaboration with Dr. Michael Lesser, University of New Hampshire, who is funded under a separate award. Dr. Lesser is collaborating in the fieldwork and is performing chemical analysis of some of the fluorescing pigments.

WORK COMPLETED

In June we successfully conducted the first exploration for fluorescence from a submersible in deep water during a 12-day research cruise in the Bahamas aboard the R/V *Seward Johnson* (Harbor Branch Oceanographic Institution). We added blue and ultraviolet filters to four 400 watt high intensity lights that we placed far forward on the Johnson-SeaLink II submersible (Figure 1). A total of 18 submersible dives were made, to depths as great as 850 m (2800 ft). We made two dives a day when conditions allowed, with the first commencing at 1200 and the second at 1930. The first dive always went to at least 350 m (1200 ft) since it was necessary to reach a depth at which it was dark enough to search for fluorescence. The night dive was usually made to shallower depths, but still below the range of SCUBA operations. Fluorescing subjects were documented in situ using the submersible's video camera fitted with appropriate barrier filters, and selected specimens were collected for spectral measurement on board ship and extraction of fluorescent pigments for analysis. In addition to the submersible work we conducted a total of 54 SCUBA dives (34 day, 20 night), exploring for fluorescence and collecting specimens for spectral measurement and pigment extraction on board ship. 122 specimen observations were added to the database.

Figure 1. View from inside the Johnson-SeaLink sphere showing the HMI lights mounted far forward on the tiltable lid of the work basket. The two inner lights are fitted with filters that transmit blue light, while the outer two are fitted with filters that transmit ultraviolet. The two pairs can be illuminated separately. To the left is the JSL video camera fitted with a yellow barrier filter for imaging blue-light stimulated fluorescence.

We completed the analysis of the contribution of fluorescence to the spectrum of fluorescent patches on a stomatopod (mantis shrimp), referenced in last year's report (Mazel, 2002). A manuscript on the use of fluorescence for signaling in this animal has been accepted for publication in Science (Mazel et al, in press).

We substantially refined the format of the spectral database and are in the process of populating it with observational information, images, and spectra. There are now over 200 entries in the database.

Extensive tests were made of the approaches to creating algorithms for automated interpretation of the fluorescence data.

RESULTS

We made the first in situ observations of fluorescence in deepwater organisms, including corals, anemones, crinoids, and others. We found that the practical requirements for searching for fluorescence were not as restrictive as originally expected. As long as the illumination spots were kept from overlapping it was possible to utilize both the blue or ultraviolet lights for fluorescence exploration and white lights for submersible operational safety at the same time. The deep-water environment at most of the dive sites was characterized by soft carbonate sediments with low density of organisms of a limited number of types, most of which did not have significant fluorescence. Brightly fluorescing animals, which were typically green-fluorescent, were easily spotted when the excitation beam passed over them.

We found that during full daylight fluorescence exploration could be conducted at depths below approximately 180 m (600 ft). At shallower depths the ambient light levels made it difficult to detect all but the brightest instances of fluorescence. The dive sites were characterized by sloping sedimented bottoms that met vertical walls at depths on the order of 300 m (1000 ft). Fluorescence was very rare on the walls until a depth of about 200 m (700 ft), at which point the orange fluorescence characteristic of encrusting phycoerythrin-containing algae began to appear (Figure 2, left). As in shallow water, the blue light filter proved more effective than the ultraviolet in stimulating fluorescence. Only a few specimens were found with the ultraviolet light that would not have been found with the blue. In addition to the deepwater observations we discovered several new instances of fluorescence in shallow-water organisms, including intense orange fluorescence in ostracods, yellow-green fluorescent amphipods, multi-color fluorescing polychaetes, and stunning magenta fluorescence in a scorpionfish (Figure 2, right).

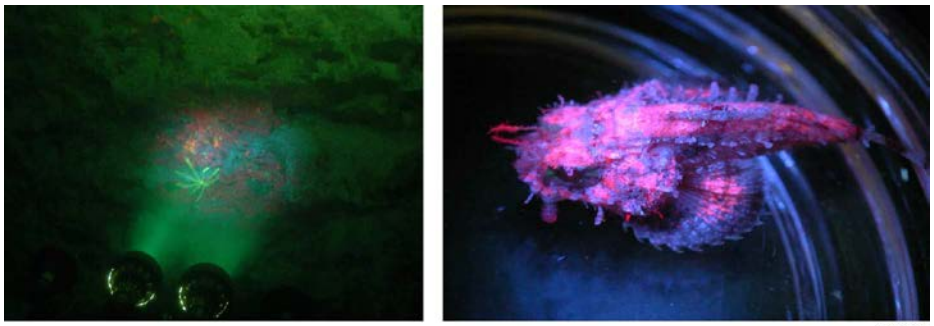

Figure 2. Left, photograph taken from inside the Johnson-SeaLink submersible showing a pair of lights directed at a vertical wall at a depth of approximately 150 m (500 ft), revealing yellow fluorescence from a crinoid and red and orange fluorescence from encrusting algae. Right, laboratory photograph showing striking magenta fluorescence in a small (6 cm) scorpionfish collected from shallow water.

Experimentation with new light sources for SCUBA diving led to the adoption of an off-the-shelf dive light with a high intensity discharge bulb as a primary tool for fluorescence exploration. When fitted with a custom blue interference filter (Figure 3) this makes an effective tool for searching for and videotaping fluorescence. This light-filter combination has become a popular commercial product offered through NightSea, a PSI subsidiary. We are currently experimenting with a new light that incorporates a high-intensity 1W light-emitting diode. The output of this light is comparable to that of the Light Cannon with filters, and has advantages such as smaller size, lower cost, and lower power consumption.

Figure 3. Underwater Kinetics Light Cannon high intensity discharge dive light fitted with a custom interference filter. The light is an effective illumination source for fluorescence exploration and close-range documentation by videotape.

For the algorithm development, we found that dividing the spectrum into as few as six equal wavelength bands would enable near-perfect recognition of the fifteen fluorescence spectral classes we modeled (Figure 4). With fewer bands the recognition capability becomes more limited. This result has significant implications for selecting detector schemes for a general-purpose multispectral fluorescence classification system.

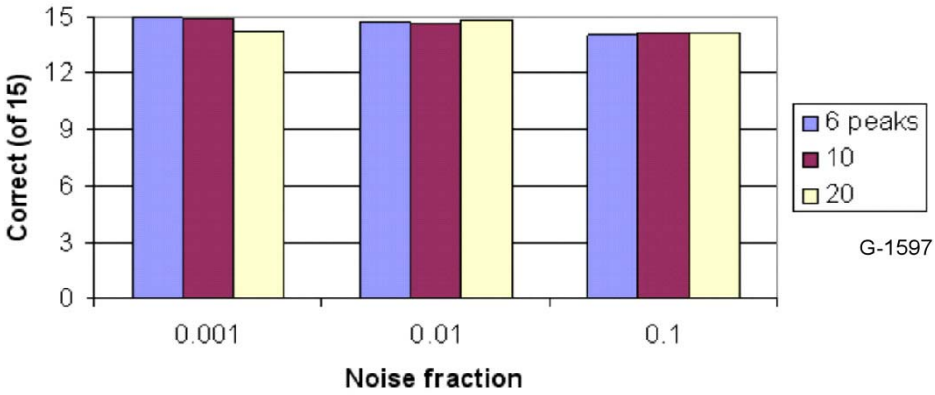

Figure 4. Bar graph showing the performance of a neural net approach in classifying the modeled spectra in fifteen spectral classes, with three levels of added noise (0.001, 0,01, and 0.1, expressed as a fraction of maximum signal amplitude). The results show that even at the highest noise level, approaches using data divided into 6, 10 and 20 evenly spaced spectral bands produced nearly identical results, all with near-perfect classification performance.

IMPACT/APPLICATIONS

The continuing discovery of fluorescence phenomena in a wider array of benthic organisms has implications for our interpretation of fluorescence imagery. In an effort funded as part of the Coastal Benthic Optical Properties (CoBOP) program (Mazel, 2003) we demonstrated the potential to use multi-wavelength fluorescence imagery generated by the Fluorescence Imaging Laser Line Scanner to perform automated classification of coral reef surfaces (Mazel et al., 2003). In that work we interpreted all brightly fluorescing pixels as being associated with corals, even for very small targets of 1 to 5 pixels. We now know that small brightly fluorescing features can arise from polychaete worms, fish, or other sources. This will not have a large impact on estimates of percent cover, but could be very important in regard to estimation of numbers of juvenile (i.e., small) corals. The current results from the algorithm development show that with several additional detection bands a fluorescence system could potentially divide scenes into finer classification groups.

TRANSITIONS

The Underwater Kinetics Light Cannon dive light fitted with the custom filters is now a standard product offered by NightSea, a subsidiary of PSI (http://www.nightsea.com/uklc.htm). The light is being used for both scientific and sport diving. On the order of 40 units have now been sold.

RELATED PROJECTS

There is overlap between this effort and continuing analysis of the fluorescence data collected as part of the CoBOP research program (Mazel, 2003). The fluorescence data interpretation results have direct application to design of a prototype fluorescence-detecting probe being built as part of another ONR effort (Neely, 2003).

REFERENCES

Mazel, C. H. 2002. Characterization of Fluorescence in the Marine Environment. ONR FY02 Annual Report.

Mazel, C. H. 2003. CoBOP Data Analysis and Research Group Coordination. ONR FY03 Annual Report, this volume.

Mazel, Charles H., Michael P. Strand, Michael P. Lesser, Michael P. Crosby, Bryan Coles, and Andrew J. Nevis, 2003. High resolution determination of coral reef bottom cover from multispectral fluorescence laser line scan imagery. Limnol. Oceanogr. 48:522-534.

Neely, J. 2003. Advanced Underwater Imaging. ONR FY03 Annual Report, this volume.

NightSea web site – Light Cannon page, http://www.nightsea.com/uklc.htm

PUBLICATIONS

Mazel, C. H., T. W. Cronin, R. L. Caldwell, and N. J. Marshall. Fluorescent Enhancement of Signaling in a Mantis Shrimp. Science [in press, refereed].